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13. ABSTRACT (Maximum 200 words)

The overall goal of this project is to develop and maintain a resource of mouse models for breast cancer research. Eleven induced mutant strains have been identified and accepted into The Jackson Laboratory (TJL) Induced Mutant Resource (IMR) repository for breast cancer research models. The importation process frees mice of infectious pathogens. Embryos are cryopreserved. Correct nomenclature has been assigned, efficient breeding strategies have been developed, and typing protocols are being modified for optimal efficiency and accuracy. Strain availability is being announced in several media, including the IMR strain list accessed through TJL's WWW home page.

A principal aim of this project is to transfer relevant mutations to defined genetic backgrounds. Many of these mutations were generated on mixed genetic backgrounds, which limits their usefulness for most genetic studies. After consultation with Associated Board members and other experts in the field, we have decided that transgenes arriving on mixed genetic backgrounds be transferred to the FVB inbred background. In addition, the transfer of tumor suppressor genes to backgrounds with defined susceptibility to mammary carcinogenesis may convey specific experimental advantages. The Trp53 and Rb1 targeted mutations are being transferred to the BALB/cJ and C3H/OuJ inbred strains.

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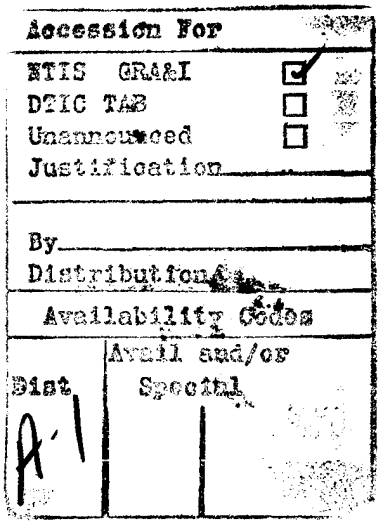

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5. INTRODUCTION

The Jackson Laboratory (TJL) is the leading world resource for both standard and special strains of inbred laboratory mice. It is a private nonprofit institution that is widely recognized for its basic research using the mouse as a model system for the study of mammalian genetics and human diseases. The Jackson Laboratory is an NCI-designated Basic Cancer Research Center, in which all research contributes directly or indirectly to understanding mechanisms of cancer, creating new mouse models for specific types of cancer, or developing therapeutic strategies. For more than 60 years, TJL has conducted research on the genetics of the mouse; developed, preserved and distributed inbred strains and mutant stocks; and educated scientists about the uses of mice in biomedical research.

The ability to add to and selectively alter the mouse genome (transgenics and targeted mutations, respectively) opens an exciting new era of research into the genetic bases of human health and disease [1-3]. Mouse mutants that provide models for specific human diseases and model systems to study the mechanisms of gene function are being produced in large numbers in many research laboratories, world-wide. The capability to design genetically engineered models for human disease is especially valuable for breast cancer research, for which no spontaneous model with an etiology identical to common human cancer types exists. Transgenic mice bearing human oncogenes or protooncogenes implicated in breast cancer have now been created by several laboratories. [4]. A wide range of targeted mutants are also now being created that will provide critical information about the role of tumor suppressor genes, growth factors, and immunologic factors in mammary tumorigenesis. Many of these mice are especially powerful tools for devising and testing novel therapeutic approaches.

The enormous potential of genetically engineered mutants can only be fully exploited if there is a central facility to preserve, propagate, and distribute them. TJL established a repository for the cryopreservation, maintenance, and distribution of important genetically altered mice (Induced Mutant Resource) in September 1992. The purpose of the project described in this report is to: 1) import and cryopreserve strains with particular relevance to breast cancer research; 2) develop rapid and accurate DNA typing methods for these mutants; 3) transfer mutant alleles to selected inbred backgrounds with defined susceptibility to mammary tumors; 4) characterize mammary tumorigenesis on those congenic strains. TJL will make these valuable mice for breast cancer research available to the biomedical research community as rapidly and freely as possible.

The specific aims of this project are to:

1. Select mutants with importance to breast cancer research for importation into the IMR. Selection involves:
   A) Identifying relevant strains
   B) Determining criteria for selection
   C) Encouraging participation by investigators holding transgenic and targeted mutants
   D) Addressing legal considerations
   E) Cooperating to avoid duplication of efforts

2. Import (by hysterectomy rederivation) transgenic and targeted mutant mice with importance for breast cancer research into defined health status breeding rooms at The Jackson Laboratory;

3. Maintain and expand breeding colonies of imported strains for cryopreservation, strain development and distribution;

4. Develop accurate and rapid methods for typing stocks for inclusion of transgenes or targeted mutations;

5. Develop improved mouse models for breast cancer research by transferring mutant genes to selected inbred backgrounds conferring specific experimental advantages;

6. Distribute mutant and control mice to scientific investigators on a cost recovery basis;

7. Maintain data on imported mutants and subsequently developed new strains in a computerized database for maintenance of nomenclature, information on mutants held in the resource and tracking information of mice.

6. BODY OF THE REPORT

SPECIFIC AIM 1: SELECT MUTANTS WITH IMPORTANCE TO BREAST CANCER RESEARCH FOR IMPORTATION INTO THE IMR.

A. Identifying Relevant Strains

Drs. Sharp and Tennent are responsible for identifying relevant strains for the breast cancer repository. Relevant strains are identified by review of the scientific literature and by consultation with TJL staff, Associated Board members Drs. Robert Coffey and William Muller, and other members of the breast cancer research community. Table 1 lists the strains accepted to the repository and offered by investigators. We pursued one additional strain, but the investigator declined to offer it until he has obtained funding to complete additional characterization.

Table 1. Strains accepted to the transgenic repository for breast cancer research

Strain designation	Type of mutation	Ref
B6D2-TgN(MMTVTGFA)29Rjc	Transgenic	[5]
FVB/N-TgN(MMTVneu)*	Transgenic	[6]
FVB/N-TgN(MMTVPyVT)*	Transgenic	[7]
STOCK TgN(MMTVTGFB1)46Hlm	Transgenic	[8]
SJL-TgN(Wnt1)1Hev	Transgenic	[9]
FVB/N-TgN(MMTVInt3)3Rnc	Transgenic	[10]
CD1-TgN(MtTGFA)42Lmb	Transgenic	[11]
FVB/N-TgN(MtTGFA)100Lmb	Transgenic	[11]
STOCK Src^{tm1Sor}	Homologous recombination	[12]
STOCK TgN(WapHRAS)69Lln	Transgenic	[13]
FVB/N-TgN(WapHRAS)69 Lln	Transgenic	[13]

* indicates symbols incomplete pending agreement with investigators on their Laboratory Registration Codes

Brief descriptions of strains accepted (from literature and personal communications with original investigators)

B6D2-TgN(MMTVTGFA)29Rjc

Transforming growth factor α is a 50 amino acid secreted polypeptide sharing 35% sequence homology with EGF. TGFα binds to the EGF receptor and is a potent mitogen for several cell types. The EGF-like family of growth factors and their receptors are prominently associated with breast cancer. TGFα promotes proliferation and formation of lobuloalveolar structures in the normal mouse mammary gland. Expression of TGFα is found in up to 70% of human breast cancer biopsy specimens and is thought to be important in early stages of mammary tumorigenesis. Because TGFα is a mitogen for mammary epithelium, it has been hypothesized to act as a tumor promoter, increasing cell proliferation and providing an environment in which cancer is more likely to result from background mutational events or carcinogenic initiators.

This transgenic stock carries human *TGFα* regulated by the mouse mammary tumor virus (MMTV) promoter, which causes selective expression in mammary tissue small ducts and alveoli in both virgin and pregnant mice. Stromal expression in some hyperplastic areas was detected by immunostaining. EGFR mRNA expression was also increased in mammary tissues expressing high levels of the transgene. Virgin transgenic mice showed no mammary gland abnormalities prepubertally, but adult virgin mice had considerable alveolar gland hyperplasia. Pregnant transgenic mice show marked proliferation of the stromal cells, and alveolar secretion is markedly increased compared to nontransgenic mice. After multiple pregnancies, isolated adenocarcinomas developed. There was no apparent phenotypic effect in males. The TGFα transgenic mice were crossed with TGFβ transgenic mice, and analysis of F1 progeny carrying both transgenes revealed marked suppression of mammary tumor formation [14]. The TGFα transgenic mice could also be used in crosses with mice carrying mutations in the EGF family to examine the differential actions of these closely related growth factors. The putative tumor-promoter effect of TGFα may also be useful in enhancing the detection of tumor-initiating events and in determining the actions of putative metastases-inducing genes.

CD1-TgN(MtTGFA)42Lmb, FVB/N-TgN(MtTGFA)100Lmb

These transgenic strains, in which human TGFα is expressed under the control of the mouse metallothionen promoter, overexpress TGFα in several tissues. On the CD1 background, zinc-treated transgenic mice showed more densely developed mammary ductal network as compared with nontransgenic animals. Dividing cells were observed in the subtending ducts as well as in the end bud region. This strain is also characterized by a high incidence of hepatocellular tumors. An FVB/N inbred transgenic strain was also generated, in which 10 copies of the transgene were inserted. This strain develops a 60% incidence of mammary tumors. These strains are both valuable for examining the effects of varying levels of TGFα expression on mammary tissue morphology, as well as the effects of background modifiers on tumor susceptibility and progression.

STOCK TgN(MMTVTGFB1)46Hlm

The transforming growth factor beta family of polypeptides have a wide spectrum of biologic effects, including involvement in mammary gland development. Originally identified for their ability to induce anchorage-independent growth of non-transformed fibroblasts, they have been found to inhibit proliferation of many epithelial cell types.

TGFβ's have an overlapping pattern of expression in mammary epithelium during branching morphogenesis, and administration of exogenous TGFβ family members inhibits ductal morphogenesis and causes the proliferating stem cell layer at end buds to disappear. They do not inhibit alveolar morphogenesis during pregnancy. The role of TGβs in cancer is complex, and may involve suppression of immune surveillance and abrogation of estrogen dependency as evidenced by effects of exogenous treatment on nude mouse--human breast cancer cell line xenograft models. TGFβ1 is normally secreted in a latent form, which can be constitutively activated by site-directed mutagenesis of cysteines in the pro region of the prepro form.

In this transgenic stock, human TGFβ1 is regulated by the MMTV promoter. Constitutively activated TGFβ1 was expressed in mammary epithelium of 13-week virgin as well as lactating animals. Expression was localized to ductal epithelium. Expression in salivary gland was also detected. Ductal development was markedly inhibited in virgin transgenic mice by 13 weeks. Epithelial proliferation was markedly reduced. Mammary glands from pregnant and lactating mice showed fully developed lobules, although transgenic mice had fewer lobules. No mammary tumors were found in transgenic mice followed for more than 300 days. These transgenic mice are useful for examining the suppressive role of TGFβ1 in mammary tumorigenesis induced by different mechanisms. For example, transgenic mice were resistant to mammary carcinogenesis induced by the chemical carcinogen 7, 12-dimethylbenz[*a*]anthracene [14]. These mice are also useful for crosses with other transgenic mice carrying oncogenes or growth factors to determine gene interactions in mammary tumorigenesis.

FVB/N-TgN(MMTVneu)

The *Neu* protooncogene encodes a 185-kDa transmembrane protein that is a member of the epidermal growth factor receptor family. Amplification and overexpression of the human homologue, *ERBB2*, is frequently (estimated 25%) observed in primary human breast cancer specimens and appears to be associated with poor prognosis. Overexpression is also found in human ovarian tumors. Overexpression of *Neu* mRNA and protein in the absence of gene amplification have also been found in several tumors. The growth-regulatory functions of *Neu* may be exerted through several different mechanisms, involving interactions with epidermal growth factor and its receptor, *Rb1*, *Myc*, and SV40 large T antigen. The transgenic stock developed by Dr. Muller's group overexpresses the protooncogene *Neu* in mammary tissue and is a model for human disease. The transgene is expressed at low levels in normal mammary epithelium, salivary gland and lung. Higher expression was detected in tumor tissue. Tumors arose as foci in hyperplastic, dysplastic mammary glands, and metastatic progression to the lung was observed in the majority of animals. There was no phenotypic effect in males. These transgenic mice can be used in crosses with other stocks carrying mutations in genes implicated in *Neu* regulation or action. They are also useful for developing and testing therapies targeted to breast tumors characterized by*Neu*- overexpression.

FVB/N-TgN(MMTVPyVT)

Infection of newborn or nude mice with polyomavirus (PyV) induces a number of tumor types including mammary adenocarcinoma. A functional middle T antigen is required for tumor induction. Middle T antigen has been shown to affect a number of signal transduction pathways, including activation of several *Src* family members.

Transgenic mice expressing PyV middle T antigen were viable and showed reduced lactational ability coincident with transgene expression. Transgene expression was detected at high levels in male and female mammary glands. Lower levels were detected in salivary

gland, seminal vesicles, ovaries, and lung (believed to originate in pulmonary metastases). Adenocarcinomas were found in virgin and breeder females as well as males. Adenocarcinomas were multifocal, highly fibrotic, and involved the entire mammary fat pad. Pulmonary metastases were observed in 80%-94% of tumor-bearing female mice. The high tumor incidence and early onset make these transgenic mice valuable models for testing preventative and therapeutic modalities, and for investigating gene interactions in mammary tumorigenesis.

STOCK *Src^{tm1Sor}*

Src is the cellular homolog of the transforming gene of the Rous avian sarcoma virus. The *Src* locus encodes a 60 kD cytoplasmic tyrosine protein kinase and belongs to a family of tyrosine kinases. *Src* is a protooncogene involved in the induction of neoplastic disease, but the normal physiological role of the gene is unknown. *Src* has been implicated in development, but its role may be masked by other tyrosine kinases. The highest levels of normal *Src* expression occur in platelets and neurons. Many members of this family have restricted patterns of expression (i.e. cells from hematopoietic lineages). Heterozygous 129/Sv-*Src^{tm1Sor}* animals are normal. The primary phenotype in homozygous mutants is osteopetrosis. In general, long bones are shorter in length and show a partial absence of bone marrow. Homozygous mutant mice are smaller (about 1/3 the weight) than normal litter-mates and incisors fail to erupt. No overt phenotype is found in brain or platelets, where it is most highly expressed.

Mutants may be used to examine the role of *Src* in signal transduction in particular experimental systems. The *Src* gene product interacts with the transmembrane protein encoded by *Neu*. *Src* null mutants may be used in combination with other targeted mutants of tyrosine kinase family members to study the normal role of this protooncogene.

SJL-TgN(Wnt1)1Hev

Wnt1, formerly *Int1*, was the first protooncogene implicated in mammary tumorigenesis by MMTV insertional activation. The *Wnt* gene family is large and is strongly conserved in evolution. The*Wnt* genes mediate cell-cell signaling events that are important for pattern formation and experimental carcinogenesis, but the mechanisms are unknown. In mice,*Wnt1* is not normally expressed in adult mammary glands, but is expressed in defined portions of the embryonic brain and is essential for the normal development of the central nervous system. *Wnt1* is also expressed in early spermatids of adult males. It has been postulated that inappropriate *Wnt1* expression in mammary glands may interfere with normal regulatory functions of other *Wnt* gene family members that are normally expressed in mouse mammary tissue, although no role for *Wnt1* homologues in human cancer has been established as yet.

Mammary glands from virgin transgenic females resembled hormonally-stimulated glands from pregnant mice. There was a marked increase in numbers of terminal branches and alveoli, producing a diffuse lobular hyperplasia. Parous transgenic females were unable to lactate. Male glands, while less developed, also were hyperplastic. Adenocarcinomas developed between 3 and 7 months in females and more rarely in males. Occasional metastatic lesions were observed in females. Salivary adenocarcinomas were also occasionally observed in both males and females. Tumors arose stochastically, indicating additional events are required for neoplasia. Subsequent work with *Wnt1* transgenic mice has included infection with MMTV to identify contributing oncogenes and mating to transgenic mice bearing other oncogenes [15]. These mice are also useful for investigating

potential autocrine or paracrine mechanisms by which *Wnt1* stimulates cell proliferation during tumorigenesis.

FVB/N-TgN(MMTVInt3)3Rnc

The *Int3* locus was identified in the wild-derived *Mus musculus musculus* strain Czech II, where it is the usual site of MMTV integration. The transcribed *Int3* sequences have significant homology to the intracellular domain of the *Drosophila Notch* gene and the yeast cell cycle regulatory genes *cdc10* and *SWI6*. Although there have been no reports of involvement of human homologs to *Int3* in mammary carcinogenesis, the human homolog of the *Notch* gene, *TAN1*, has been shown to be translocated in acute T-lymphoblastic leukemia.

Mammary glands of virgin female transgenic mice showed incomplete glandular development, with reduced ductal penetration of the fat pads and absence of end buds. Parous transgenic females failed to lactate. Ductal penetration was more extensive than for virgin transgenic mice, but lobular-alveolar development was incomplete. Hyperplastic nodular lesions were observed at multiple sites, and multifocal adenocarcinomas developed as early as 7 weeks of age. Pulmonary metastases were observed. Salivary glands also showed hyperplasia and adenocarcinoma development, while Harderian/lacrimal glands, and nasal mucosal/submucosal glands showed hyperplasia. Males were sterile and were characterized by hyperplastic and disorganized pseudostratified epididymal epithelium. Sperm count was occasionally reduced. This is a model for metastatic mammary carcinoma.

STOCK TgN(WapHRAS)69Lln, FVB/N-TgN(WapHRAS)69Lln

The 3 *RAS* oncogenes, *HRAS*, *KRAS*, and *NRAS*, encode 21-kD proteins called p21s. Mutated *HRAS* genes are found in a variety of human tumors and are powerful transforming agents *in vitro*. The TgN(WapRAS) transgenic lines carry an *HRAS* with a Gly to Val mutation at codon 12 which was originally derived from T24 human bladder carcinoma cells. *HRAS* expression under the control of the mouse whey acidic protein (Wap) promoter is tissue specific and hormone dependent. The transgene integrated into the Y chromosome. These transgenics serve as models for male mammary cancer, RAS-mediated solid tumors and the transgene also can be used as a Y-chromosome marker. In the STOCK background males develop multiple mammary tumors and salivary gland tumors by one year of age. The salivary tumors are adenocarcinomas arising from serous areas of the submandibular gland. The mammary gland tumors are adenosquamous carcinomas. Microscopic lung metastases were present in 14% of tumor-bearing animals. On the FVB/N background males develop multiple mammary tumors at 6-8 weeks. The tumors are adenosquamous carcinomas with multiple foci of squamous differentiation or adenocarcinomas containing glandular tissue. There is no metastases observed. Strain differences in susceptibility to metastasis may be useful for identifying genes involved in malignant progression. The original investigators have submitted a paper examining the effect of crossing this transgene onto other backgrounds.

B. Criteria for Selection

Drs. Sharp and Tennent present identified strains to the Genetic Resources Committee, chaired by Dr. Davisson, for a decision regarding selection. Criteria for selection of mutants is based on existing guidelines for importing mice to the Laboratory's Genetic Resources. These are: 1) the immediate need for use in biomedical research; 2) the numbers of requests for mice being received by the investigators who created them; 3) the potential for future research; 4) the time and effort needed to replace or recreate the mutant;

and 5) the uniqueness of the mutation. When more than one stock exists for mutations at the same locus, the IMR either imports the stock deemed to most closely resemble the analogous human disease or import selected mutants that can be used to relate mutations in specific parts of the gene to specific aspects of the disease. Transgenic stocks carrying the same introduced gene may have significantly different phenotypes depending on the insertion site, copy number and promoter used. For all mutants, the phenotype of the mutation, physical nature of the mutation, and genetic background are used to assess the relative potential of duplicative mutations for productive research.

C. Encouraging Participation by Investigators Holding Transgenic Mice

Several approaches are being used to expand the current level of contributions to the IMR. These include notices of the IMR in general and specific notices about the breast cancer repository. Many of the mutant stocks submitted to the IMR through these mechanisms are relevant to breast cancer research. 1) An IMR presentation is given at all courses and workshops held at The Jackson Laboratory. These courses include the *Short Course in Medical and Experimental Mammalian Genetics*, given annually in association with the Johns Hopkins University School of Medicine; *Experimental Genetics of the Laboratory Mouse*, a graduate and post-graduate level course led by an international faculty; the *Cryopreservation* course and special workshops and conferences focused on animal models; 2) IMR personnel accept all relevant speaking and writing opportunities to disseminate information and invite participation in the program. Drs. Tennent and Sharp announced the formation of a breast cancer repository within the IMR at the July 1994 Press Week, held in conjunction with the *Short Course* at TJL and attended by 20 science reporters for national publications. An article has appeared in *Lab Animal* [16], Dr. Muriel T. Davisson was an invited speaker at a *Workshop on Genetically Engineered Animal Models* and Dr. Sharp was an invited speaker at the *Living Cultures Collection Workshop* organized by the Genetic Society of America and NSF. Dr. Sharp presented at a March of Dimes workshop on disease models. In 1994 a poster session was given at the *Ninth International Workshop on Molecular Genetics of the Mouse* and we plan to submit a poster to at least 2 scientific meetings in 1995, including the Annual Symposium on Fundamental Cancer Research to be held in Houston, Texas in October. 3) In February 1995 all users of JAX mice (a mailing list of 7000) received a list of all accepted IMR strains, together with information concerning submission of strains to the IMR. This list will be mailed annually and list updates will be made through posting in *JAX Notes*, a quarterly publication mailed to the same recipients. This list is also available through The Jackson Laboratory home page on the World Wide Web (WWW; http://www.jax.org/). We have begun to post the genetic typing protocols for each strain on the WWW as well. 4) Dr. Carol Linder, Technical Services Advisor for TJL, distributes information about the IMR program at selected scientific meetings where The Laboratory trade booth is exhibited. 5) Announcements and inventories are posted on TJL's World Wide Web home page, including the statement that the IMR seeks new mutants. 6) Announcements are being placed in *Nature*, *Nature Genetics*, *Cancer Research*, and *Immunology Today* to notify holders of induced mutant animals that the IMR will cryopreserve and distribute their animals. These journals have an international circulation and/or serve disciplines which develop transgenic mice frequently. A tracking system using individual response numbers and "Bingo Cards" permits us to evaluate the effectiveness of each journal in reaching potential donors and users. The results are now being compiled and an optimal notification system will be established. 7) The IMR will continue the very successful effort to encourage participation by approaching investigators holding mice of interest. This informational program also widely distributes information on available stocks to potential research users.

The IMR has a significant impact on research as evidenced by the increased distribution of IMR strains each month. Monthly distribution from the IMR as a whole has increased approximately 300% in the past year and approximately 2200 mice were distributed in May 1995. Awareness about the IMR increases demonstrably following publications and mailings. A continuing effort to keep the scientific community aware of IMR developments in general and the breast cancer repository in particular will be crucial. Approaches both to increase awareness about the breast cancer repository and to contact investigators holding mice of interest have been successful, as illustrated in Table 2.

Table 2. Summary of strains requested or offered to the IMR's breast cancer repository

# Requested from Investigator	# Offered by Investigator	# Accepted by GRC	# Declined by Investigator
7	7	12*	1

* Two strains offered within the reporting period will be considered at the July, 1995 GRC meeting

D. Addressing Legal Considerations

TJL has formulated a general approach to enter negotiations, based on over two years' experience. TJL encourages participation in the IMR program and attempts to discourage institutions from imposing licensing or royalty requirements. When necessary, however, distribution agreements have been negotiated requiring royalty payments by the Laboratory and/or notices to for-profit companies that a commercial license may be required from the originating institution. The Jackson Laboratory, as a policy matter, will not be a party to any distribution agreement that restricts the ability of the investigator to breed animals for research purposes, or restricts the Laboratory's ability to distribute mice on a first come-first served basis. We have succeeded in maintaining these principles because of our reputation in the scientific community. The legal negotiations at The Jackson Laboratory are the responsibility of David Einhorn, Esq., The Jackson Laboratory in-house counsel. Seven strains accepted to the breast cancer repository this year, including three strains from the National Cancer Institute, have required that agreements be negotiated. Agreements have been reached with NCI and one other institution. Negotiations continue with a private company holding two strains. These negotiations are time-consuming and can delay the distribution of stocks. We expect legal considerations to continue, although we expect that negotiations can be more quickly completed as originating institutions acquire experience.

E. Cooperating to Avoid Duplication of Efforts

We have found that duplication of effort is best avoided by contact with the investigator holding the mice requested. Investigators who initiate the contact usually have offered their mutants only to The Jackson Laboratory. To screen for potential duplication, a form is sent to each potential provider of mutant animals asking if the animal is being offered to other institutions. Investigators are also asked for their knowledge of any similar animal being produced elsewhere. Investigators holding mice relevant for breast cancer research are very helpful in suggesting mice for the repository and discussing the specific experimental advantages of similar models.

Information exchange between institutions providing similar services should also be maintained. There are few restrictions to this information exchange among non-profit or academic institutions. Contacts and collaborations already exist between The Jackson Laboratory's Scientific Staff and many institutions and individuals who might also be involved in similar efforts. The IMR's National Advisory Board and associated members are also well-connected in the mouse genetics community and their direction helps reduce duplication. The exchange of information with for-profit service providers is more limited. Nevertheless, The Jackson Laboratory continues to communicate with other institutions or organizations who also cryopreserve and distribute transgenic and/or targeted mutant mice.

SPECIFIC AIM 2. IMPORT (BY HYSTERECTOMY REDERIVATION) TRANSGENIC AND TARGETED MUTANT MICE WITH IMPORTANCE FOR BREAST CANCER RESEARCH INTO DEFINED HEALTH STATUS BREEDING ROOMS AT THE JACKSON LABORATORY

Mice from the STOCK-*Src*^{tm1Sor} strain have been released from importation. A strain carrying the *Src* null allele on a 129 inbred background is also currently in the importation facility. B6D2-TgN(MMTVTGFA)29Rjc, STOCK TgN(MMTVTGFB1)46Hlm, STOCK TgN(WapHRAS)69Lln, FVB/N-TgN(WapHRAS)69Lln, and SJL-TgN(Wnt1)1Hev strain mice are currently in importation. Strains for which legal negotiations are completed are scheduled for importation.

SPECIFIC AIM 3. MAINTAIN AND EXPAND BREEDING COLONIES OF IMPORTED STRAINS FOR CRYOPRESERVATION, STRAIN DEVELOPMENT AND DISTRIBUTION

Linda Washburn, Senior Professional Assistant, is manager of the Induced Mutant Resource Colony (IMRC). Upon importation of a mutant stock, a foundation colony is established, from which embryos or sperm for freezing are obtained. The colony is expanded to provide mice for strain development and distribution. Strains in continuing demand are distributed from a breeding colony. If requests for a strain fall below one per year the breeding colony may be discontinued and the stock maintained only as frozen embryos; a decision is based on an evaluation of the strain's research importance and the availability of the strain through other sources (such as its maintenance in TJL or other research colonies that could supply limited numbers per year). Strains for which there is a large demand are expanded in the Induced Mutant Production Colony (IMPC), maintained by Animal Resources. For such strains a foundation breeding nucleus is maintained in the IMRC to provide pedigreed breeders to the Production colony.

SPECIFIC AIM 4. DEVELOP ACCURATE AND RAPID METHODS FOR TYPING STOCKS FOR INCLUSION OF TRANSGENES OR TARGETED MUTATIONS

The IMR allele typing program is the responsibility of Dr. Sharp. Virtually all mouse strains in the IMR require genetic typing to confirm the presence of the transgene in transgenic strains, or the genotype of targeted mutants. Genetic typing is required to identify carrier animals (heterozygotes) for strains being backcrossed onto a defined genetic background (in excess of 90% of all strains), to identify hemizygous animals for those transgenic strains supplied as hemizygotes, and to identify heterozygotes for those strains where the homozygous mutants are embryonic lethals or do not reproduce. Allele typings are carried out using the polymerase chain reaction (PCR) because it is rapid, the reaction conditions may be standardized, it does not require the use of radioisotopes, and it is adaptable to automation.

Allele typing protocols for IMR strains are first developed in the IMR Development Laboratory under the supervision of Valerie E. Scott, Professional Biomedical Technologist. Ms Scott is responsible for developing and testing all genetic typing protocols for each new strain and is also responsible for overseeing the correct use of these protocols. She has contacted all of the researchers supplying mice to the breast cancer repository and is optimizing protocols for the strains already received. She reviews all typing results and provides these protocols to researchers requiring assistance. The protocols are regularly posted on the World Wide Web.

Automation of the allele typing procedures, using the fluorescent based PCR detection system of the Applied Biosystems division of Perkin Elmer (ABI), is under development. An ABI Model 373A Automated DNA Sequencer equipped with the Genotyper software and an ABI biomedical robot have recently been purchased to automate allele typing procedures.

SPECIFIC AIM 5. DEVELOP IMPROVED MOUSE MODELS FOR BREAST CANCER RESEARCH BY TRANSFERRING MUTANT GENES TO SELECTED INBRED BACKGROUNDS CONFERRING SPECIFIC EXPERIMENTAL ADVANTAGES

Because of the marked strain differences in susceptibility to spontaneous, hormonally-induced, and chemically induced mammary adenocarcinomas and the number of as yet unidentified "background" modifying genes (including MMTV proviral insertions) participating in susceptibility, it is essential to place transgenes and targeted mutant genes on defined, inbred backgrounds. Appropriate selection of mutant alleles and inbred backgrounds will increase the utility of these models for breast cancer research. Strains accepted to the breast cancer repository this year include several different inbred or segregating backgrounds. This genetic heterogeneity essentially precludes the use of these stocks for intercrossing to determine cooperativity among mutant genes in mammary carcinogenesis. Five of the mutations are maintained on an FVB/N inbred background. Our Associated Board members and several other investigators holding breast cancer models strongly suggest that other mutant genes arriving on heterogeneous backgrounds be transferred to the FVB/N inbred strain. FVB/N mice are used by many investigators to make transgenic mice, because of the exceptionally large pronuclei, good reproductive performance [17], and the lack of milk-transmitted MMTV or replication-competent endogenous MMTV proviruses. Most of the mouse models for breast cancer we have identified to date are transgenic strains, although targeted mutant models will likely be generated as tumor suppressor genes participating in mammary carcinogenesis are identified and cloned. If the original investigator agrees, we will transfer mutant genes to the FVB/N inbred strain by repeated backcross. We are aware that the phenotype can change or be lost as backcrossing progresses. We use several measures to prevent the loss of phenotype. 1) The original stock is maintained while backcrossing progresses. 2) As a precaution, we routinely cryopreserve 200 embryos from the original stock as soon as possible after the stock is imported. At the fifth generation of backcrossing, matings will be expanded as needed, carrier females will be identified and examined for tumor development. If the tumor phenotype is preserved, backcrossing will then be resumed.

Selected transgenes may also be transferred to the C57BL/6J (B6) inbred background. Several of the strains accepted to the breast cancer repository this year are maintained on hybrid backgrounds with a B6 component, and inbreeding to B6 would be advantageous. Most strains imported to the larger IMR program are routinely backcrossed to B6, so that a large number of mutations valuable for examining gene interactions and mechanisms of immune recognition are already available on this standard background. The C57BL genetic

background is commonly used by immunologists, and many experimental tools are available for use with this background.

Cooperativity with strain-characteristic susceptibility alleles may also be established by transferring mutant genes to different inbred backgrounds with defined susceptibility to mammary carcinogenesis. We are transferring the *Trp53* [18] and *Rb1* null mutations [19] to the BALB/cJ (*Trp53*, N = 4; *Rb1*, N=3) and C3H/OuJ (*Trp53*, N=4; *Rb1*, N=4) backgrounds. Matings will be expanded at N5 and progeny will be examined for increased incidence and shorter latency to mammary carcinogenesis. The BALB/cJ strain was chosen because spontaneous papillary adenocarcinomas with histomorphology closely resembling the human infiltrating ductal carcinoma have been observed in the TJL production colony of this strain [20]. The C3HOu/J strain was chosen because mice develop a high frequency of MMTV-induced mammary adenocarcinomas and are widely used in breast cancer research.

SPECIFIC AIM 6. DISTRIBUTE MUTANT AND CONTROL MICE TO SCIENTIFIC INVESTIGATORS ON A COST RECOVERY BASIS

The informational program described in Specific Aim 1, Section D, also widely distributes information to potential users. The IMR is clearly an important resource to the biomedical research community, as evidenced by the increasing monthly distribution figures. In May 1994, approximately 700 mice were distributed and by May 1995, approximately 2200 were distributed. Strains are made available on a minimal or limited basis as soon as they have cleared importation. *Src^{tm1Sor}* strain mice are being distributed on a limited basis while the breeding colony is expanded to support wider distribution and the collection of embryos for cryopreservation.

SPECIFIC AIM 7. MAINTAIN DATA ON IMPORTED MUTANTS AND SUBSEQUENTLY DEVELOPED NEW STOCKS IN A COMPUTERIZED DATABASE FOR MAINTENANCE OF NOMENCLATURE, INFORMATION ON MUTANTS HELD IN THE RESOURCE AND TRACKING INFORMATION OF MICE

The IMR database was developed by Dr. Sharp to track internal management information on all IMR strains. It is maintained in FoxPro and is currently limitedly accessible to IMR and Genetic Resources personnel. The IMR database contains strain information such as gene description, phenotype, husbandry, typing methods, and nomenclature. All the genetic databases at The Jackson Laboratory use correct nomenclature following the guidelines of the International Committee on Standardized Genetic Nomenclature for Mice. When strains are accepted for importation to the breast cancer repository, Drs. Sharp or Tennent suggest appropriate nomenclature with the original investigator, get approval from the Mouse Genome Database Nomenclature Coordinator, and arrange for assignment of a laboratory code, as necessary. Strain information from the IMR database is routinely posted on the World Wide Web, accessible through the Jackson Laboratory's home page. Information is also transferred to the Informatics group at TJL for inclusion in the Mouse Genome Database (MGD), which is maintained at TJL and is available on the WWW.

7. CONCLUSIONS

Eleven induced mutant strains of particular relevance to breast cancer research have been identified and accepted for importation into The Jackson Laboratory Induced Mutant Resource repository for breast cancer research models. Correct nomenclature has been assigned to each strain, an important step for disseminating information about mutant strains and reducing duplication of effort. Efficient breeding strategies for each strain have been developed, and typing protocols have been obtained and are being modified for optimal efficiency and accuracy. The availability of these strains is being announced in several media, including the IMR strain list accessed through The Jackson Laboratory's WWW home page.

The need to negotiate agreements for seven of the strains accepted to the IMR under this program has delayed the importation and distribution of some strains. We expect that the time required for negotiations will decrease as the transfer of mutants to the IMR becomes more routine and the originating institutions clarify their objectives. In the 02 year of this project, we will also include in our planning an increased time estimated for legal negotiations, so that importation to the IMR can be scheduled in a timely manner and the objectives of this grant to import ten strains per year can be met.

Many induced mutant stocks of particular relevance to breast cancer research have been created and maintained on mixed genetic backgrounds, which limits their usefulness for most genetic studies. At present, many of these models for breast cancer are transgenic stocks carrying oncogenes or growth factors with expression directed to the mammary gland. From literature surveys and discussions with breast cancer researchers, it appears that most transgenic mice are generated using either hybrid fertilized eggs or eggs from FVB/N inbred mice. Our Associated Board members and other experts in the field have recommended that transgenes arriving on mixed genetic backgrounds be transferred to the FVB/N inbred background. The Genetic Resources Committee of The Jackson Laboratory has accepted this recommendation.

The breast cancer research community is increasingly aware of the transgenic repository for breast cancer research supported by the US Army Breast Cancer Research Program. When the project began, we identified mutants through literature searches and contacted individual researchers holding mice of interest. Now investigators are beginning to offer strains for consideration by the Genetic Resources Committee, and are telling us about strains they are currently developing and plan to offer in the future. The need for this repository is compelling. We look forward to importing, preserving, developing, and distributing a variety of valuable models for breast cancer research in the coming years.

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APPENDIX I:

List of personal receiving pay:

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Richard Smith, Pathologist

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